



COMMUNICATIONS IN SOIL SCIENCE AND PLANT ANALYSIS
Vol. 34, Nos. 15 & 16, pp. 2193–2201, 2003

A Sensitive Method for the Measurement of Ammonium in Soil Extract and Water

Hojeong Kang,^{1,*} Emily H. Stanley,¹ and Seok-Soon Park²

¹Center for Limnology, University of Wisconsin,
Madison, Wisconsin, USA

²Department of Environmental Science and Engineering, Ewha Institute of Science and Technology, Ewha Womans University, Seoul, Korea

ABSTRACT

Ammonium is one of the most important inorganic nutrients in many ecosystems. The utility of a sensitive method for the measurement of ammonium (NH_4^+) in water, soil and plant-derived samples using fluorescence of an *o*-phthalaldehyde (OPA)-based compound was evaluated in this study. This method involves less toxic chemicals than a conventional method, requires minimal equipment, and allows for fairly rapid sample processing. This method is not only sensitive for low (e.g., sub-micromolar) NH_4^+ concentrations, but also applicable for samples with much higher concentrations ($> 100 \mu\text{M}$ NH_4^+) by using a modified spectrophotometric protocol developed herein. This evaluation demonstrates that this method provides a valuable tool for the measurement of

*Correspondence: Hojeong Kang, Department of Environmental Science and Engineering, Ewha Institute of Science and Technology, Ewha Womans University, Seoul 120-750, Korea; Fax: 82-2-3277-3275; E-mail: hjkang@mm.ewha.ac.kr.

NH_4^+ in a variety of environmental samples including soil extracts, litter leachates and highly polluted water.

Key Words: Fluorescence; Nitrogen; Methodology; Eutrophication.

INTRODUCTION

Ammonium (NH_4^+) in aquatic and terrestrial ecosystems is of great interest to many researchers because nitrogen is often a limiting nutrient and NH_4^+ is an easily assimilable form of this nutrient for microbes or plants. At high concentrations, NH_4^+ becomes toxic, and thus is a pollutant of increasing importance in many ecosystems.^[1] However, reliable and safe methods for NH_4^+ analysis have been elusive.^[2,3] The most widely used methods for measuring NH_4^+ in soil or water involve an indophenol blue compound.^[4,5] Although such methods are used for various types of samples, several shortcomings have been noted.^[5,6] These include: high and variable blanks; a detection range (typically $0.01\text{--}1\text{ mg L}^{-1}$)^[7] that may not be sensitive enough for low concentrations (e.g., sub-micromolar) characteristic of many aquatic ecosystems; and use of toxic chemicals (i.e., phenol, sodium nitroprusside) that require special disposal. As an alternative approach, a fluorometric technique using o-phthaldialdehyde (OPA) has been introduced for samples from marine and freshwater ecosystems.^[3,8]

Ecological studies frequently examine large areas with several different components (i.e., watershed or landscape scale studies) and consequently generate a range of sample types with a concomitant diversity of nutrient concentrations. For example, one project might involve collection of relatively dilute water samples (e.g., from an oligotrophic lake), soil extract (e.g., KCl extract from forest floor soils), stem flow, or litter leachate. It is also common to generate a large number of samples at a time in a single environmental study. Thus, a method that could accommodate such a wide range of sample types and concentrations would offer obvious advantages.

For a method to be effective, the following requirement should be satisfied. First, the method should be sensitive to a wide range of NH_4^+ concentrations from sub-micromolar to over 100 micromolar (i.e., less than 0.01 mg L^{-1} to over 1 mg L^{-1}). Second, the method should exhibit minimal interference with any extractants or other chemicals in the samples. Finally, the method should not involve complicated equipment, protocols or settings (e.g., HPLC) so that it can be used widely and turnover rate could be reasonably high. The protocol developed by Holmes et al.^[3] has been shown to meet most of these requirements but only for water samples. The specific aims of this study were to

**Measurement of NH_4^+ in Soil Extract and Water****2195**

determine if this method could be used to analyze samples of terrestrial origin, including leaf leachates and KCl-based soil extracts, and to present a modification of the technique applicable to NH_4^+ rich samples.

MATERIALS AND METHODS**Method Principles**

The method developed by Holmes et al.^[3] is based on quantifying the fluorescence of the reaction product of NH_4^+ and OPA. From our investigations of the robustness of this method for terrestrial samples, we offer a modification that broadens the method's application. At high NH_4^+ concentrations ($> 10 \mu\text{M}$), a dark orange color appears, presumably due to a reaction between NH_4^+ and the reagent. This orange color interferes with the fluorogenic measurement by quenching the fluorescence. However, we observed that the color intensity increases as the concentration of NH_4^+ increases. Thus, high nutrient concentrations can be measured by shifting from fluorometric-based to spectrophotometric-based determinations.

Reagent preparation for our analyses followed the methods described in detail by Holmes et al.^[3] Fluorescence was determined using a Turner Designs TD-700 fluorometer equipped with optical Kit No. 10-303 (UV mercury lamp, 350 nm excitation filter, and 410 to 600 nm emission filter). Two protocols were considered, depending on sample concentration.

Protocol I (fluorometer-based) can detect two concentration ranges ($0.000\text{--}0.718 \mu\text{M}$ and $0.00\text{--}7.18 \mu\text{M}$) simply by changing the sensitivity of the fluorometer. For both settings, 1 mL of sample is combined with 4 mL of the working reagent. The sample/reagent mix was incubated in the dark at room temperature for 2 hours, followed by measurement of fluorescence. Protocol II (Spectrophotometer) is appropriate for higher concentrations ($0.0\text{--}71.8 \mu\text{M}$). The same procedures as Protocol I were applied to samples except for the measurement of color absorption at 415 nm using a 1-cm cell in a Beckman DU-640 spectrophotometer instead of the fluorometer.

Effects of Reaction Conditions

NH_4^+ determination for plant or soil-derived samples could be affected by several reaction conditions and interferences, such as incubation time, the ratio between sample and working reagent volumes, interference with dissolved organic carbon, or interference with KCl solution. For the OPA

method to be useable for plant and soil samples, it should be relatively insensitive to variation in these conditions. Therefore, we conducted a series of experiments to assess possible interferences and effects of methodological variation. First, the fluorescence of reactants (using a $3.59 \mu\text{M}$ NH_4^+ sample) was determined every 30 minutes for 3 hours. Second, ratios between sample and the working reagent volumes were varied 0.25, 0.67, 1.5, or 4 (again using a $3.59 \mu\text{M}$ NH_4^+ sample). Third, different concentrations of a dissolved organic carbon (DOC) solution were added to samples to determine quenching or matrix effect on fluorescence or absorption. A highly colored DOC solution was prepared by leaching 10 g of leaf litter (fresh *Acer saccharinum*) in 50 mL deionized water for 24 hours followed by filtration through a $0.2 \mu\text{m}$ filter. A series of DOC amendment solutions with concentrations of 0, 178.5, 666.7, 750, 1500 μM were made by serial dilution of the leachate. Three mL of each DOC solution was added to 1 mL of NH_4^+ solution ($7.18 \mu\text{M}$), and the concentration was determined following Protocol I. Fourth, we determined if standard curves were strongly linear for standards made with 1 M KCl. For each experiment, at least three replicates were employed, and the differences among treatments were sought using one-way ANOVA test, if applicable (i.e., experiments of DOC addition and variations in the sample/reagent ratio).

Application

The method developed in this study was applied to three types of samples: leaf litter leachates, KCl-based soil extracts, and water samples collected from a constructed wetland containing high concentration of ammonium (higher than 2 mg L^{-1}).

Fresh leaf litter was collected from three trees: silver maple (*Acer saccharinum*), basswood (*Tilia americana*), and swamp white oak (*Quercus bicolor*) from the Wisconsin River floodplain, Sauk County, WI (USA). Soil samples were collected under the canopy of each tree to assess between-tree variation in soil NH_4^+ content. The litter leachates were prepared as described above, and soil samples were extracted with 1 M KCl solution followed by filtration through a $0.2 \mu\text{m}$ filter. NH_4^+ concentrations of both litter leachates and soil extract were determined following the protocols described above.

The spectrophotometric protocol was used to analyze water samples collected from a constructed wetland in Seoul, Korea. The constructed wetland (ca. 1.5 ha; mean depth is 0.5 m) is operated to remove inorganic nutrients in effluent from a water treatment plant. Inflow and outflow samples were collected in August 2001 on 9 occasions. Samples were filtered with

Measurement of NH_4^+ in Soil Extract and Water**2197**

a 0.2 μm filter followed by incubation with the reagent as describe above. The water samples were not diluted, and color intensity was measured with a spectrophotometer following the protocol II.

RESULTS AND DISCUSSION

Calibration curves prepared for the two different protocols exhibited good linear relationships ($r^2 = 0.998$ and 0.999 , $P < 0.001$). A calibration curve prepared with 1 M KCl solution showed lower fluorescence, but still had significant linearity ($r^2 = 0.988$, $P < 0.01$). The calibration curve for the spectrophotometric method also showed a significant linearity ($r^2 = 0.997$, $P < 0.001$) (Fig. 1).

Fluorescence emitted from the reactants reached maximum values after 1 hour and was maintained for 3 hours (Fig. 2). Therefore, a 2-hour incubation was employed for all subsequent experiments. In evaluating the effects of the ratio between the amounts of a sample and the reagent, a 1:4 (sample: reagent) ratio produced the highest fluorescence of the ratios that were considered (Fig. 3). This means that only small amounts of sample (as little as 1 mL) are needed for this analysis. When samples were spiked with the DOC solution,

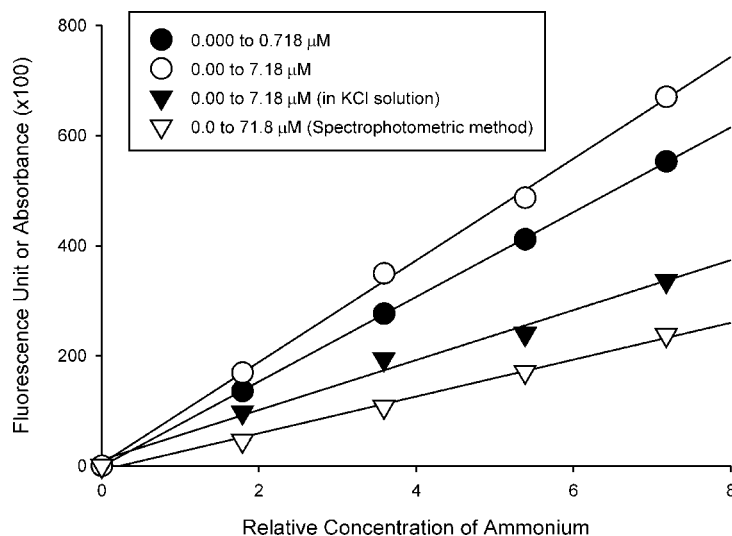


Figure 1. Calibration curves for the NH_4^+ measurement.

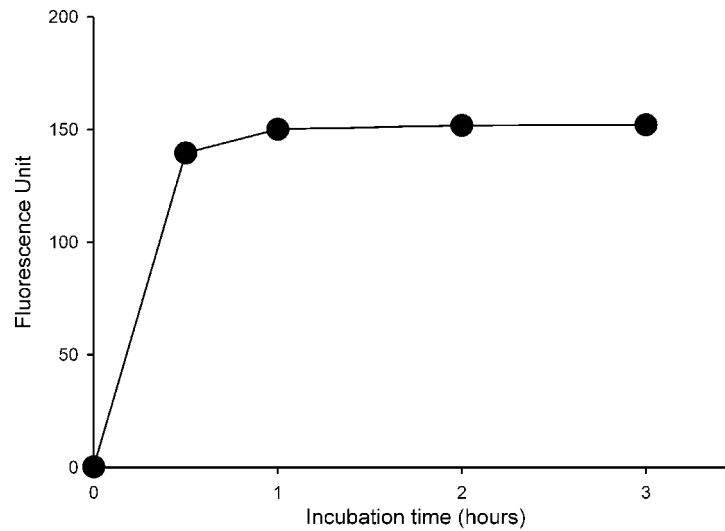


Figure 2. Effects of incubation time on the measurement.

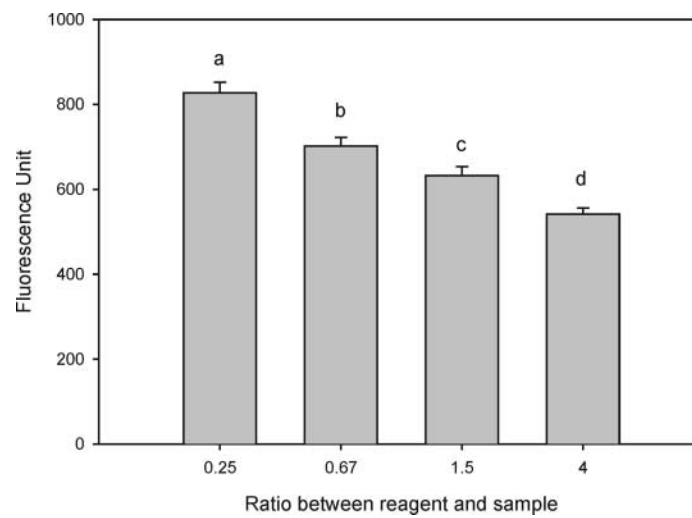


Figure 3. Effects of the ratio between sample and reagent. Data labeled with different letters are significantly different at $P < 0.05$ (one-way ANOVA followed by Tukey's test). The percentage values on the above of bars represent relative values compared to the control as 100%.

Measurement of NH_4^+ in Soil Extract and Water

2199

the intensity of fluorescence decreased, likely due to quenching effects of the DOC (Fig. 4). However, the decrease in fluorescence was only significant when the concentration of DOC added reached 1500 μM (One-way ANOVA, $P < 0.01$). Further, this interference only caused a 10% decrease of the fluorescence emitted from the control (Fig. 4). This indicates that interference with DOC is negligible, except for samples with extremely high colored DOC levels—such as might come from bog-dominated systems. In such cases, it may be appropriate to dilute samples to reduce this quenching effect. However, samples with extremely low concentration of NH_4^+ but excessively high background color from DOC present a serious challenge to the present method, just as it does for other conventional methodologies (e.g., indophenol method). Accurate determination of NH_4^+ for such samples might be possible by using HPLC to separate out DOC materials,^[9,10] but would come at the cost of high sample turnover rates.

It was possible to analyze NH_4^+ content and detect species-specific differences for 'typical' samples derived from the Wisconsin River floodplain. Leaf leachate prepared from the three different tree species contained significantly different amounts of NH_4^+ (Table 1). Highest concentrations were found in the leachate from basswood, followed by silver maple and

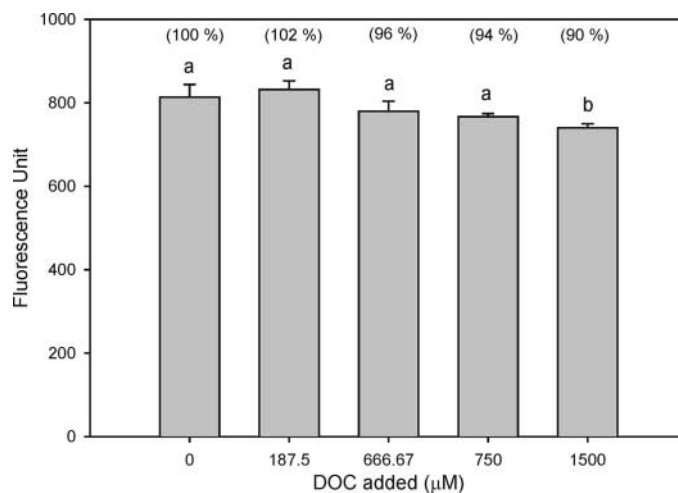


Figure 4. Effects of dissolved organic carbon interference on the measurement. Data labeled with different letters are significantly different at $P < 0.05$ (one-way ANOVA followed by Tukey's test).

Table 1. Application of the *o*-phthaldialdehyde method to various types of environmental samples. Data labeled with different letters are significantly different at $P < 0.05$. Values in the parentheses are standard error of the means.

Sample type		Concentrations ($\mu\text{mole g}^{-1}$ or $\mu\text{mole L}^{-1}$)	Detection types
Soil KCl-extract	<i>A. saccharinum</i>	535 (13.4)	Protocol I (fluorometer)
	<i>T. americanan</i>	477 (52.1)	
	<i>Q. bicolor</i>	435 (52.6)	
Leaf litter leachate	<i>A. saccharinum</i>	2.3 (0.2) ^a	Protocol I (fluorometer)
	<i>T. americanan</i>	4.0 (0.3) ^b	
	<i>Q. bicolor</i>	0.6 (0.1) ^c	
Water in a constructed wetland	Inflow	37.3 (4.0) ^a	Protocol II (spectrophotometer)
	Outflow	14.2 (2.7) ^b	

swamp white oak. However, extractable NH_4^+ in soil was not significantly different among soils under different types of tree canopy.

The results from a constructed wetland exhibited a significant decrease in ammonium concentration between inflow water and outflow water ($P < 0.05$, paired sample t-test,). The average concentration of ammonium in inflow water was $37.3 \mu\text{M}$, while the concentration in outflow water was $14.2 \mu\text{M}$, indicating that the constructed wetland is of value for the removal of inorganic nutrients such as ammonium.

The measurement of NH_4^+ has been troublesome for many scientists,^[2] which may partly be due to the rapid changes in NH_4^+ in the samples as well as the inaccuracy of conventional procedures. Overall results of this study suggest that the OPA method^[3] and the modifications proposed herein are applicable to various types of environmental samples with substantially different characteristics. In particular, the protocol proposed here would be of value to assess soil samples with both extremely low and extremely high NH_4^+ concentrations.

ACKNOWLEDGMENTS

This research was funded by the Environmental Protection Agency STAR Program (Grant No. R826600). The completion of the manuscript was

**Measurement of NH_4^+ in Soil Extract and Water****2201**

supported by a grant (No. 5-4-1) from Sustainable Water Resources Research Center of 21st Century Frontier Research Program endowed to H. Kang and S-S. Park.

REFERENCES

1. Carpenter, S.R.; Caraco, N.F.; Correll, D.L.; Howarth, R.W.; Sharpley, A.N.; Smith, V.H. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* **1998**, *8*, 559–568.
2. Aminot, A.; Kirkwood, D.S.; K  rouel, R. Determination of ammonia in seawater by the indophenol-blue method: evaluation of the ICES NUTS I/C5 questionnaire. *Mar. Chem.* **1997**, *56*, 59–75.
3. Holmes, R.M.; Aminot, A.; K  rouel, R.; Hooker, B.A.; Peterson, B.J. A simple and precise method for measuring ammonium in marine and freshwater ecosystems. *Can. J. Fish Aquat. Sci.* **1999**, *56*, 1801–1808.
4. Solorzano, L. Determination of ammonium in natural waters by the phenolhypochlorite method. *Limnol. Oceanogr.* **1969**, *14*, 799–801.
5. Catalano, G. An improved method for the determination of ammonia in seawater. *Mar. Chem.* **1987**, *20*, 289–295.
6. Bower, C.E.; Holm-Hansen, T. A salicylate-hypochlorite method for determining ammonia in seawater. *Can. J. Fish Aquat. Sci.* **1980**, *37*, 794–798.
7. Eaton, A.D.; Clesceri, L.S.; Greenberg, A.E. *Standard Methods for the Examination of Water and Wastewater*, 19th Ed.; American Public Health Association: Washington, DC, 1995.
8. K  rouel, R.; Aminot, A. Fluorometric determination of ammonia in sea and estuarine waters by direct segmented flow analysis. *Mar. Chem.* **1997**, *57*, 265–275.
9. Freeman, C. Using HPLC to eliminate quench interference in fluorogenic substrate assays of microbial enzyme activity. *Soil Biol. Biochem.* **1997**, *29*, 203–205.
10. Kang, H.; Freeman, C. Measurement of phosphomonoesterase activity in a wetland sediment: a sensitive method using HPLC and UV detection. *Arch. Hydrobiol.* **1997**, *140*, 411–417.